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Human Bocavirus in Hospitalized Iranian Adults with Respiratory Tract Infections during January-June 2014

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The epidemiology of respiratory human bocavirus (HBoV) infection has not been described in Tehran's adult and to determine the epidemiological and clinical characteristics associated with HBoV infection, a population of adults hospitalized with respiratory tract infections were chosen. Throat swab samples were collected from 91 hospitalized adults aged between 29 to 91 year and Real-time PCR TaqMan was used to screen specimens by amplifying a part of the NP1 gene. HBoV was detected in 6 adults (6.6%). Mean age was 76 years and 67% of patients were female. The most common symptoms were wheezing (100%), tachypnea (100%), cough (100%), rhinorrhea/pharyngitis (83.33%) and fever (83.33%) which clinically diagnosed by a physician. Gastrointestinal symptoms was present only in 1 patient (16.6%). In our study the distribution of HBoV was influenced by temperature, relative humidity and precipitation. HBoV is circulating in Tehran and is associated with both upper and lower respiratory tract disease in adults.

Key words: Respiratory Infections, Adults, Human Bocavirus, Real-time PCR.

Etiology of respiratory tract infections is diverse and several novel respiratory viruses are continuously being reported. New respiratory viruses such as Human Metapneumovirus, Coronaviruses NL63 and HKU1, SARS Coronavirus and Human Bocaviruses have been discovered during the past decade. The availability of molecular diagnostics assays and using conventional virological diagnostic techniques has importantly increased our ability to detect and characterize the epidemiology of respiratory virus infections¹⁻⁵.

Human bocavirus belongs to the genus *bocavirus*, family *parvoviridae*, and is a non-enveloped, round (19-22 nm) and icosahedral in

symmetry with a small genome size of 5.3 kilo-bases (kb). Genome itself has 3 ORFs encoding two non-structural proteins (NS1, NP1) and two structural proteins (VP1, VP2)^{6,7}. The virus exists in 4 different serotypes, unlike HBoV1 which has been primarily detected in respiratory samples⁸⁻¹⁰, the newer viruses (HBoV2, 3, 4) have been found frequently in human stool samples^{8,12,13}. Parvoviruses are capable of systematic infections, in order to replicate they need proliferating host cells, so infection of respiratory and gut epithelium, hematopoietic cells and transplacental infection of fetuses are frequent characteristics of this family. Therefore they can be associated with respiratory tract infections¹³. Subsequently HBoV was reported in 1.5% - 8.3% of respiratory samples with acute respiratory tract illness (ARTI) from different countries and regions worldwide^{9-11,14-18}. Primary infections with HBoV happens early in life

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(children between 6-24 month) and these infections are rarely found in adults¹⁹⁻²⁰. Detection of HBoV mostly relies on classical and Real-time PCR²¹⁻²³ on nasopharyngeal aspirates and swabs^{19,21,24-27}.

The current study aims to screen the epidemiological and clinical status of HBoV isolates in adult patients suffering from acute and lower respiratory tract illness in Tehran, Iran.

Patients and Specimen Collection

During winter and spring (January to June 2014) 91 samples were collected from adults hospitalized with respiratory infections. The cross-sectional study population aged between 29 to 91 years. All specimens were collected from Tehran's hospitals with informed consents from the patients during their hospital stay and as the part of the diagnosis, pneumonia, bronchiolitis, asthma, acute nasopharyngitis, chest retraction were diagnosed by the physician through physical examinations and chest X-Rays. Patients with immune-compromised conditions like chemotherapy or immunosuppressive therapy, bone marrow transplantation were all excluded from this study. Throat swab specimens were collected from each patient and stored in a viral transport medium. Samples were transported to the virology laboratory of Iran University of Medical Sciences immediately and stored at -70°C until use. All patients' medical records, demographic and clinical data including detailed signs and symptoms and laboratory results were collected.

Nucleic Acid Extraction

Viral nucleic acid extraction was performed in a "clean room" facility within the medical virology department (Iran University of Medical Sciences), by using an Invisorb® Spin Virus DNA mini Kit (Stratec molecular, Germany) according to the manufacturer's instructions. After extraction, the elution volume (100µL) stored at -20°C for subsequent analyses.

Real-Time Polymerase Chain Reaction

The Real-Time PCR assay with hydrolyses TaqMan Probe was performed using a commercial, Real Q-PCR 2x Master mix Kit (Amplicon, Denmark) in Bio-Rad CFX96. Real-Time PCR primer and Probes were as follows:

Forward primer: 5'-AGA GGC TCG GGC TCA TATCA-3'

Reverse primer: 5'-CAC TTG GTC TGA GGT CTTCGAA-3'

Probe: 5'-FAM-AGG ACC ACC CAA TCARCC ACCTAT CGTCT-TAMRA-3'

Where FAM is 6-carboxyfluorescein and TAMRA is 6-carboxytetramethylrhodamine. The amplification reaction mixture was conducted by 4µL of extracted specimen, 10µL TaqMan universal PCR master mix (PE applied bio systems), 0.1µL bovine serum albumin (20_{mg/ml}), 300_{nmol/liter} of each primer and 150_{nmol/liter} of Boca probe. After 15 min at 95°C, 40 cycles of amplification (95°C for 20_{sec} - 55°C for 20_{sec} - 72°C for 40_{sec}) were performed. HBoV positive samples were confirmed.

Statistical analysis

Comparisons were made by using Fisher's exact test for 2x2 tables and T-Independent tests. All test's P-value < 0.05 were considered statistical significant.

RESULTS

1. Demographic characteristics of patients: The HBoV testing was performed on throat swab specimens which was collected during respiratory infections from 91 hospitalized adults with an age ranging from 29 to 91 years. Since the seasonal distribution of HBoV was noted during the winter and spring and HBoV is detectable less frequently in adults, data are scarce among the elderly. This study's seasonal distribution is demonstrated in Fig1 which shows HBoV to be predominant in March. The male to female ratio of the 6, HBoV positive adults was 2:4 and all 6 patients were older than 60 years of age (Table 1).
2. Clinical and Laboratory Findings: The clinical characteristics of the patients are listed in Table 2 which illustrates, most patients have symptoms of upper respiratory tract illness (URTI) including cough (100%), rhinorrhea (83%) and in less frequently there were symptoms of lower respiratory tract illness (LRTI) including pneumonia (67%), bronchiolitis and bronchitis (50%) which were diagnosed by chest radiography and sputum culture requested by physician. Table 3 represents clinical and laboratory findings of all patients in HBoV positive and Negative groups. Alanine aminotransferase (ALT),

Table 1. Demographic data of adults suffered from respiratory distress in the current study

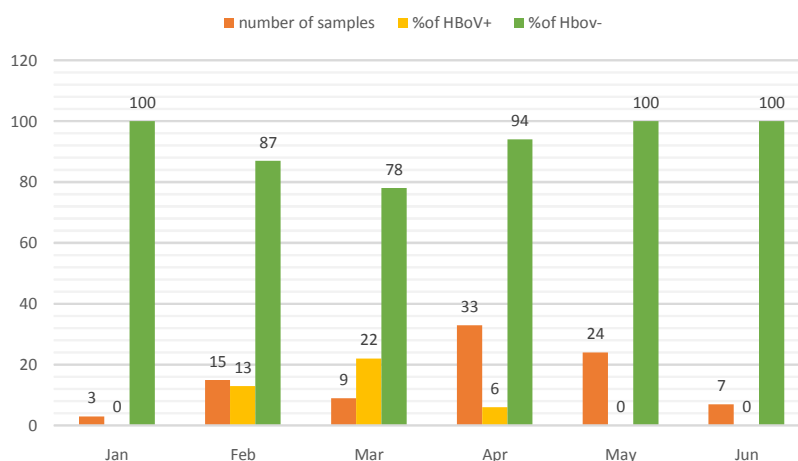
Variable	Value	HBoV infected
Age	28-91 yr	
28-41 yr	7	0
42-51 yr	6	0
52-61 yr	15	0
62-71 yr	16	2
72-81 yr	29	2
82-91 yr	18	2
Gender		
Male	51	2
Female	40	4

Table 2. Symptoms and clinical diagnosis among 6 episodes associated with HBoV infection

	N(%)	P-value
Fever>38.5°C	5 (83.33 %)	.672
Cough	6 (100 %)	.021*
Rhinorrhea/Pharyngitis	5 (83.33 %)	.672
Shortness of breath	4 (66.66 %)	.649
Gastrointestinal symptoms	1 (16.66 %)	.672
Asthma	2 (33.33 %)	.642
Bronchiolitis and bronchitis	3 (50 %)	.651
Pneumonia	4 (66.66 %)	.649
Wheezing	6 (100 %)	.656
Chest retraction	2 (33.33 %)	.152
Tachypnea	6 (100 %)	.610
Neurological disorder	4 (66.66 %)	.554

Table 3. Clinical and laboratory findings of patients

Clinical data	HBoV+(n=6)	HBoV-(n=85)	P-value
Age	75.83 ± 9.23	68.32 ± 15.79	0.255
White blood cells	12066.66 ± 9625.52	12396.47 ± 20160.44	0.969
Neutrophils (%)	83.33 ± 9.6	77.44 ± 10.5	0.187
Lymphocytes (%)	12.83 ± 7.3	17.79 ± 9.1	0.182
Platelet count (/mm ³)	197500 ± 117974	238156.47 ± 92497.19	0.309
C-reactive protein (mg/dl)	1.66 ± 1.03	4.58 ± 13.13	0.59
SGOT(IU/I)	20 ± 8.36	30.51 ± 23.08	0.272
SGPT(IU/I)	19.16 ± 8.61	31.61 ± 47.58	0.526
ESR(mm/hr)	10.83 ± 6.99	16.17 ± 12.20	0.294
Albumin (g/dl)	2.95 ± 0.13	3.82 ± 6.54	0.746
Gender (female %)	66.66	42.35	0.399
Neurologic disorder (%)	6.3	93.7	0.554
Wheezing (%)	6.2	93.8	0.656
Chest retraction (%)	5.8	94.2	0.152
Tachypnea (%)	6.1	93.9	0.61

**Fig. 1.** Monthly distribution of HBoV from January to June 2014

Aspartate aminotransferase (AST), ESR, platelet count in both groups were within the normal ranges; while White-Blood-Count (WBC), Neutrophils, C-reactive protein (CRP) and albumin were higher and lymphocytes were less than normal ranges. Frequency of wheezing and tachypnea were significantly higher in HBoV positive group and all 6 patients had these 2 symptoms. 2 of HBoV positive patients had chest retraction and 4 of them had neurological disorders.

Beside these findings, there was a significant difference (according to the P-value) between cough symptom among HBoV positive and negative patients ($P=.021$).

DISCUSSION

Human bocavirus is one of the new emerging viruses for the past decade which was first described by Allander *et al* in 2005 , since that time lots of studies occurred throughout the world and only a few ones have described the characteristics of HBoV infection in elderly^{11,19,24,25}. This study evaluate the characteristics of HBoV positive adults suffered from respiratory tract illness with a wide age distribution from Tehran,Iran for the first time.

In our study all of the patients with HBoV infections were older than 62 years of age as Liu *et al* also demonstrated the same data in china³² which suggested that old people are also susceptible to HBoV infection but data are scarce on the occurrence and consequences of HBoV infection among the elderly^{11,19,24,28,33-38}.

As most studies found that peak occurrence of HBoV infection are throughout spring and winter^{6,15,21,25,27-30,33-36,39-45} on this cross-sectional study we focused on these 2 seasons to collect samples and the peak for HBoV infection was in April. Yet gender specific differences have not been reported⁴⁶.

Previous studies have implicated HBoV as a cause of RTI. With respect to the clinical features of HBoV infection , there were 8 major symptoms among HBoV positive group including cough ,fever > 38.5% , wheezing , tachypnea , neurologic disorder , pneumonia , shortness of breath ,

rhinorrhea/pharyngitis which had the highest ratio (50%) in compared with the HBoV negative group (Table 2) . Also in several studies it has been mentioned that the frequent occurrence of lower respiratory tract symptoms especially wheezing indicates HBoV maybe a significant cause of asthma exacerbation^{23-25,29-31,43,47-54}. It must be considered that the presence of HBoV in airway epithelium may not be able to interpret its role as a pathogen and co-infection with other viruses in elderly are common. therefore serology is suggested to be a useful diagnostic in addition to the study of HBoV infection because of high seroprevalence rate against HBoV in adult population^{13,55}, also detection of HBoV viremia , viral loads in quantitative PCR are more useful prophesiers of HBoV associated clinical disease than only qualitative detection of HBoV DNA in respiratory tract samples .

In conclusion, the present study provided a first insight into the epidemiology and clinical aspects of HBoV infection in hospitalized adults in Tehran. Our findings indicate disease associated with HBoV range from pharyngitis to bronchitis, asthma and even pneumonia and in seasonal pattern with HBoV cases peak months are within January and June.

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